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ORIGINAL PAPER

Development of cork oak (*Quercus suber* L.) seedlings in response to tree shelters and mulching in northwestern Tunisia

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Abstract: The need for reforestation in cork oak (*Quercus suber* L.) areas is challenged by difficulties. Principal among these is herbivory of young plants, vegetative competition, and slow growth rates of cork oak seedlings. We evaluated the early development of cork oak seedlings treated using tree shelters and mulching in northwestern Tunisia. We tested three tree shelter treatments (non-vented, vented, and control) to shield seedlings from animal damage and five mulch types to control competing vegetation (Italian Stone Pine, Lentisk, combination of Italian Stone Pine and Lentisk (organic mulches), gravel (inorganic mulch) and no mulch). At the end of the two-year experiment, sheltered seedlings were 89-99% taller than unsheltered seedlings and had higher numbers and lengths of shoot growth flushes. In contrast, both stem diameter growth and dry weight biomass (from samples extracted after two years) were significantly reduced inside tree shelters. Root-to-shoot ratio was not significantly different in sheltered vs. unsheltered seedlings, suggesting that tree shelters do not adversely affect this parameter. Mulching alone did not favour growth, but could be beneficial when combined with tree shelters. The combination of vented tree shelters and gravel mulch was the most effective treatment for promoting diameter, height and stem volume growth.

Keywords: *Quercus suber* L.; afforestation; tree shelter; mulching; seedling growth; polycyclism

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Introduction

Cork oak (*Quercus suber* L.) forests are one of the most important natural resources in many Mediterranean countries and are of special concern because of their unique ecological and socio-economic functions (Chaar et al. 2008). In Tunisia, cork oak forests are mainly located in the northwest region where local inhabitants graze livestock. They are valuable reservoirs of biodiversity and play a key role in the maintenance of other natural resources, such as soils, air and water. The selling of their products represents about 60% of the forest returns (Nsibi et al. 2006). In addition, the range of products and services offered by the species, such as cork, firewood, acorns and landscape diversity, ensure cork oak a privileged position amongst forest species in Tunisia. Cork oak is a slow-growing species, however, and regeneration is presently not adequate to maintain cork oak populations in many areas. In Tunisia, natural regeneration is traditionally relied upon in cork oak stands but, unfortunately, natural regeneration success is low due to factors including high seed predation, ageing, cork oak decline and adverse climatic conditions. Seedling planting, as an alternative to natural regeneration, has also been relatively ineffective due to overgrazing (Hasnaoui 1992), vegetative competition and slow growth of seedlings during the early stages (Chaar et al. 2008).

The Tunisian State has long attempted to overcome regeneration failures by using very high planting densities and forbidding grazing access to reforested areas for a period of 10–15 years

until trees are established. Local inhabitants generally do not comply with grazing bans, however, because the fenced off areas deprive them of grazing land. Among the techniques that have shown promise for establishing oaks (*Quercus* spp. L.) in other regions is the use of individual seedling browsing protectors made of a translucent plastic-based material, i.e., tree shelters (Tuley 1985; West et al. 1999; Taylor et al. 2006), which could be an alternative to fencing. In addition to their protective role from browsing, tree shelters also promote seedling survival (Potter 1991) and height growth (Burger et al. 1992) by changing the atmospheric environment seedlings are exposed to, i.e., CO_2 concentration, air temperature, relative humidity and vapor pressure deficit (Potter 1991; Bergez and Dupraz 2000; Oliet and Jacobs 2007). However, tree shelters may cause negative effects in both diameter and biomass growth (Burger et al. 1996; Mayhead and Boothman 1997; Puértolas et al. 2010) before seedlings emerge from the shelters (Famiani et al. 2007).

Many researchers have examined the effect of tree shelters on growth of a range of broad-leaved tree species in Mediterranean climates often characterized by water stress, excessive radiation and high temperature during summer (Costello et al. 1996; Bellot et al. 2002; Oliet et al. 2005; Puértolas et al. 2010). In this climate, plant response to tree shelters seems to be species-specific, with many species showing improved growth when grown with tree shelters (Leroy and Caraglio 2003; Oliet et al. 2005; Puértolas et al. 2010). However, seedling damage and stress may occur because of the temperature increase inside airtight tree shelters, highlighting the importance of ventilation holes (Mayhead 1992; Bergez and Dupraz 2000).

In addition to protection offered by tree shelters, weed control strategies are needed to facilitate hardwood seedling establishment. Mechanical (cultivation), biological (cover producing companion species), chemical (herbicide) and mulching are the major types of weed control employed (Truax and Gagnon 1993; Löf et al. 2012). The latter has gained considerable popularity in tree plantations due to its ability to increase the availability of key soil resources such as nitrogen (Truax and Gagnon 1993) and water (Haywood and Youngquist 1991), and to conserve soil moisture over long periods (Huang et al. 2008), which in turn enhances plant growth and survival (Davies 1988a; Greenly and Rakow 1995). Mulches can be either organic or inorganic (Duryea et al. 1999). Organic mulches are heterogeneous in both the tree species and plant parts (leaves, branches, wood and bark) used, while inorganic mulches include polyethylene film, pebbles and gravel.

Past studies investigating the growth response of oak seedlings to either tree shelters or vegetative competition control (Burger et al. 1992; Burger et al. 1996; McCreary and Tecklin 1997; Navarro Cerrillo et al. 2005) were often limited to height, diameter and biomass measurements. However, like other oak species (Reich et al. 1980; Champagnat et al. 1986; Harmer 1990), cork oak has a rhythmic pattern of height growth (Alatou 1990). Multiple shoot growth flushes can occur in the same growing season in response to favorable growth conditions (Chaar et al. 2008). During each shoot flush, a distinct shoot portion called the growth unit (GU) is established along the main stem (Chaar and Colin 1999). The study of the periodic height growth pattern of cork oak involves division of the main stem into GUs and gives useful information on this growth response. Most previous experiments examining the growth response of oak seedlings to tree shelters, excepting Navarro Cerrillo et al. (2005), did not study the impact of tree shelter ventilation on seedling growth. Few studies have assessed the combined effect of tree shelters and mulching on tree establishment (Dubois et al. 2000; Navarro Cerrillo et al. 2005).

Our objective was to test the effectiveness of tree shelters and mulch for cork oak forest restoration. We evaluated (1) the effects of tree shelters and mulch on different growth traits and on polycyclism, i.e., tendency for multiple flushes within one growing season and (2) the effectiveness of ventilation, i.e., several holes at the bottom of tree shelter, on early growth and biomass attributes in a Mediterranean cork oak (*Q. suber* L.) plantation. We tested the following hypotheses: (1) tree shelters, especially those designed with holes as a ventilation system, promote growth and survival of cork oak seedlings; (2) mulching has an additional positive effect on seedling growth.

Materials and methods

Experimental site

The experiment was carried out at the M'hibeus National Forest (9°07′52″N, 37°06′05″E, elevation 200 m a.s.l.) in northwest Tunisia (Sejnène forest subdivision). The climate is Mediterranean with annual mean temperature of 18.2°C (1975–2004). Maximum and minimum temperatures average 34.4°C and 5.6°C, respectively. Average annual rainfall is 912 mm, with 77% of total precipitation occurring in autumn and winter and only 4% in summer. The soil at the study site showed a balanced texture in horizon A, clay loam in horizons B and C (Table 1). Soil organic matter content was 5.74%, 2.43% and 0.78% in horizons A, B and C, respectively. Prior to planting, the site was cleared of maquis vegetation mainly dominated by *Calycotome villosa* L., *Cistus monspeliensis* L., *Myrtus communis* L. and *Pistacia lentiscus* L.

Plant material

In February 2009, 300 one year old (1-0) seedlings were hand planted on the site at 4 × 4 m spacing. Seedlings were grown in a local Sejnène nursery in 2000 cc containers, filled with *Acacia cyanophylla* Lindl. bark compost. Prior to planting, all vegetation in each planting grid line was cut close to ground level. Manual hoeing around each planted seedling created an even surface of 1 m^2 for the mulch sheeting. The prepared surface around each seedling was 3 cm deep to properly affix the mulch and secure it from wind or runoff. Seedlings were watered immediately after planting with 5 L per seedling. Any dead seedlings were replaced 2 weeks later. The experimental site was fenced with wires held up by 2.5 m wooden stakes to restrict herbivore access.

Table 1. Soil properties of the study site collected at 0–150 cm depth

Soil depth (cm)	Chemical properties							Granulometry		
	OM (%)	C (%)	P ($mg{\cdot}L^{-1}$)	K ($mg{\cdot}L^{-1}$)	pH	CEC (mS/cm)	Water saturation (%)	Clay (%)	Silt (%)	Sand (%)
0–42	5.74	3.33	28.36	141	6.55	0.405	45	22.2	28.84	27.76
42–64	2.43	1.41	18.13	-	5.55	0.358	45	53	23.59	11.85
64–150	0.78	0.45	5.85	108	5.51	0.43	60	54.11	23.75	14.38

Experimental design and treatments

A split-plot design was used with Whole Plots in Randomized Blocks with four replications (blocks). A set of 75 seedlings per block were distributed for planting and allocated between the following treatments: five mulch and three tree shelter types, with 5 replicates for each type. Seedlings were planted in a rectangular block (64 × 24 m) following a spatial pattern distribution (15 columns × 5 rows). Mulch types were the whole-plots and tree shelter types were the subplots. Surrounding each whole plot, a one-row buffer strip was planted to minimize edge effects between treatments. Tested mulches were: 1) Italian Stone Pine (*Pinus pinea* L.); 2) Lentisk (*Pistacia lentiscus* L.); 3) combined mulch of Italian Stone Pine and Lentisk (50–50%) (organic mulches); 4) gravel (inorganic mulch) and 5) untreated control (no mulch). Organic mulches consisted of prunings, 20 to 40 cm long and 5 to 20 mm diameter. They were not oven-dried before use due to the risk of their rapid disintegration during the primary rainfall season, which could lead to accelerated decomposition (Oelbermann et al. 2004). Moreover, use of fresh biomass is more representative of natural decay processes at the soil surface (Fang et al. 2008). Inorganic mulch consisted of gravel (calibration: 4-16 mm) purchased at a gravel quarry in Bizerte. Davies (1988b) found that the mulch benefit was correlated to the size of the sheet, and recommended a minimum of 1 m^2 size for optimal results. Consequently, mulch materials were applied in an approximately 3 cm thick layer to an area of 1 × 1 m square around individual seedlings (Fig. 1). The three tree shelter types were: (1) 'Non-vented' tree shelter, (2) 'Vented' tree shelter, and (3) Control with no shelter. The non-vented tree shelters (Tubex 'L' Standard®) were translucent green, circular, 1.8-m tall, 8.0−12.0 cm wide, UV stabilized polypropylene with twin-walls (Tubex Co., South Wales, UK). Tree shelter walls were airtight so fresh air entered only through the top of the tree shelter (Fig. 1). Vented tree shelters (Tubex 'E' Equilibre®) were ventilated by ten 1-cm-diameter round holes at their base, creating a ''chimney effect''. Tree shelters were buried 5 cm into the soil immediately after seedlings were planted to prevent air movement through the shelter base that would desiccate the seedlings. Tree shelters were then secured to 1.8 m untreated eucalyptus stakes anchored 20 cm into the soil. Mesh caps were placed over tree shelters to exclude birds.

Growth measurements

Observations were made of all seedlings except border seedlings. Seedling height and basal diameter were recorded immediately after planting, with measurements ranging from 28.6 to 105 cm, and 3.1 to 11.6 mm, respectively (mean height 65.1 ± 0.71 cm; mean basal diameter 7.5 ± 0.08 mm). Seedling height and basal diameter were similar for all treatments. Height and basal diameter were recorded again at the end of the 2009 and 2010 growing seasons, along with mortality. As survival was very high (98%, only 6 of 300 seedlings died), treatments were not analyzed for survival differences. Heights were recorded from the base of the seedling to the end of the longest shoot. Diameter measurements were taken approximately 2 cm above the ground. An indicator of stem sturdiness was calculated by dividing height by basal diameter. Stem volume was obtained from height and diameter measurements using the following formula: stem volume (cm^3) = ((basal diameter)^2 × height × π/12). Browsing was also recorded during survival assessments.

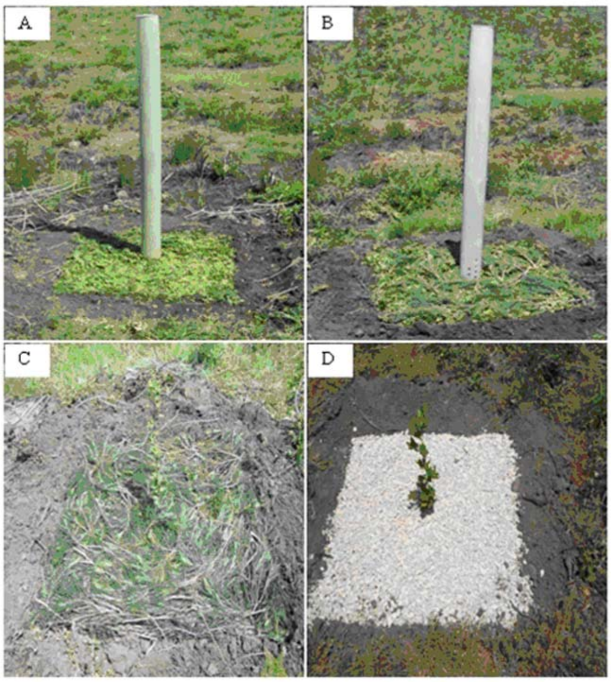

Fig. 1 Organic (A, B and C) and inorganic (D) mulches and non-vented (A) and vented (B) tree shelters: sheltered seedling (non-vented tree shelter) mulched with Lentisk (A); sheltered seedling (vented tree shelter) mulched with combination of Lentisk and Italian Stone Pine (B); unsheltered seedling mulched with Italian Stone Pine (C) and unsheltered seedling mulched with gravel (D). All mulches were 1 m^2 and approximately 3 cm thick.

After tree shelters were removed, each protected seedling's ability to support itself without a stake (its posture) was evaluated by determining whether the seedling would stand on its own (erect posture) or bend and touch the ground (bent posture).

GUs are composed of nodes, on which are inserted basal scales or foliage leaves, and internodes (Chaar et al. 1997). GUs occurr during a given growth season from a portion of the stem called the annual shoot (Fig. 2). The following growth components were recorded each growing season: length and number of internodes per GU, number of flushes established by the main stem (polycyclism rate), GU length for each flush, and annual shoot length. The shoot elongation period (growth flush) as well as the rest period was also determined.

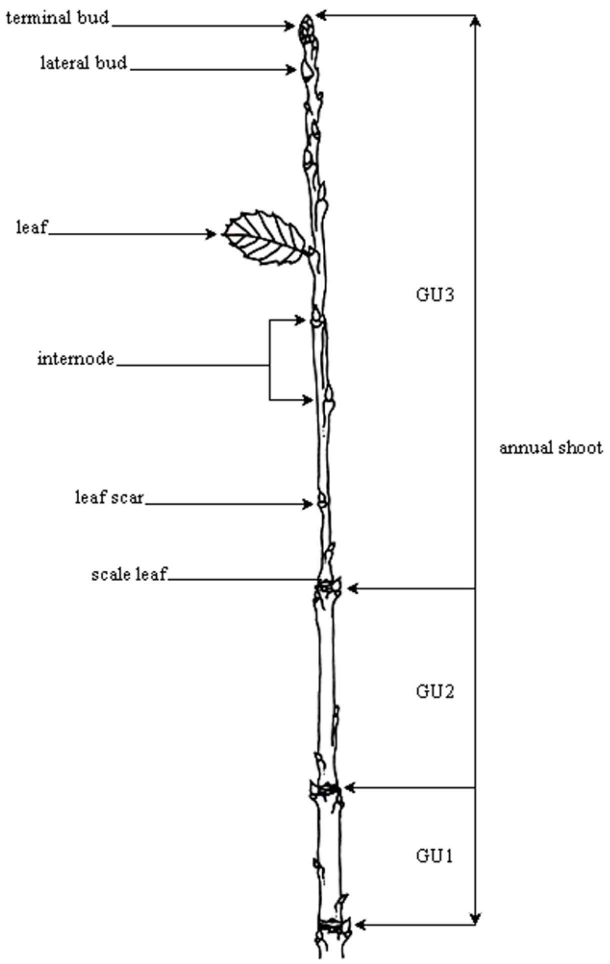

Fig. 2 Structure of a cork oak annual shoot: an example of a tricyclic annual shoot (three growth units (GU)).

In December 2010, 5 seedlings randomly selected from each treatment were harvested to determine seedling biomass. Leaves were removed from stems, stems were severed at the root-collar, and all roots were then rinsed free of soil. Weight of the different fractions of above- and below-ground biomass (leaves, branches, stem and roots) were then measured after drying at 80°C for 5 days. Root-to-shoot ratio was calculated for each seedling from dry weights of the biomass components. Leaf area and leaf mass per area were also recorded. The leaf area of each seedling was calculated using a scanner and computer image analysis system (WinFolia Basic). The leaf mass per area (LMA) was determined after removing 30 cm^2 of leaf tissue, which was dried at 80 °C for 48 h and then weighed.

Temperature measurements

Daily maximum and minimum temperatures inside tree shelters were measured using a maximum-minimum thermometer (Mercury free max-min thermometer) suspended by monofilament fishing line at 50 cm height above soil level. Four tree shelters of each type were randomly chosen and equipped with a thermometer. Temperatures recorded inside tree shelters were then compared with measurements similarly recorded with four thermometers on the control treatment. The measurement period was June-August 2010.

Statistical analysis

Final height, basal diameter, height-to-diameter (*H/D*) ratio, and stem volume of seedlings were analyzed as a split–plot arrangement with whole plots in randomized blocks and with repeated measures. Growing season (2009 and 2010) was treated as a repeated measures effect; the initial value of the variable (measured just after planting) as a covariable; the two factors Tree shelter type and Mulch type and their interactions as fixed effects; and Block, Block × Mulch type as random effects. Analysis of covariance (ANCOVA) was then conducted (Littell et al. 2006). Heterogeneous AR (1) was chosen as a covariance matrix (Littell et al. 2006) to accommodate heterogeneous variances over time. Annual shoot length was analyzed with the additional effect of the covariate: number of flushes per growing season. AR (1) covariance model was selected. GU length for each flush was analyzed by introducing into the model the additional effects of both flush number (1^{st}, 2^{nd} or 3^{rd}), number of flushes (1, 2 or more than 3), and possible interactions with other factor effects.

Data from destructive sampling and temperatures were analyzed using analysis of variance (ANOVA). Seedling posture (bent or erect) was a binary variable and was analyzed using a logistic linear model (Agresti, 2002):

$$\text{logit}(p_1) = \log\left(\frac{p_1}{1-p_1}\right) = \beta X$$

where p1 is the rate of mortality or of bent seedlings. X is a column vector of explanatory fixed variables and β is a column vector of unknown coefficients.

Probabilities (= $p_1/(1-p_1)$) and probability ratios were calculated and tests of significance performed using the ESTIMATE option of the GENMOD Procedure. The probability ratio expresses the probability that a certain event is the same for two factor levels. A ratio of 1 implies that the event is equally likely for both levels. A ratio >1 implies that the event is more likely in the first level and a ratio <1 implies that the event is less likely.

We used a cumulative logit model to analyze the ordinal vari-

able, number of flushes (1, 2 or more than 3). We modeled the probabilities of response levels having lower ordered values in the response profile table. That is, seedlings producing one or two flushes, with $p1$ and $p2$ proportions, respectively, were compared with those producing three flushes ($p3$) in the resulting probability (($p1$ + $p2$)/$p3$). The proportional probability was tested. Likelihood confidence intervals at $\alpha = 0.05$ were calculated for the proportions.

Statistical analyses were performed using PROC MIXED and PROC GENMOD procedures of SAS Version 9.1 (SAS Institute Inc., Cary, NC). Comparisons between treatment means and proportions (percentages) were made using the Tukey-Kramer multiple comparison test and Chi^2 test, respectively, with an entry and exit significance of $P \leq 0.05$.

In no case was the effect of the block or its interaction with the studied factors significant.

Results

Temperature

Air temperature differed significantly inside versus outside (control) the tree shelters, both for maximum ($p < 0.0001$) and minimum ($P = 0.0002$) daily temperatures. Overall, maximum temperatures were higher inside than outside tree shelters during the entire period of measurement (Fig. 3). Maximum temperatures inside non-vented tree shelters (40.13°C) were, on average, 8°C warmer than that outside. Ventilation of tree shelters significantly reduced the difference between the air temperatures inside and outside tree shelters by an average of 5°C. During the entire period of measurement, maximum temperatures inside non-vented, vented and outside tree shelters reached, 51, 45.5 and 42.5°C, respectively. Contrary to maximum temperatures, minimum temperatures inside non-vented and vented tree shelters were, on average, 2 and 1°C less than outside (21.37°C) tree shelters, respectively. Average minimum temperature outside tree shelters was not significantly different from that recorded inside vented tree shelters.

Stem height

Stem height ranged from 50 to 274.8 cm in 2009 (mean: 136.2 ± 3.1 cm) and from 55 to 280 cm in 2010 (mean: 156.7 ± 3.5 cm). Some of the smaller seedlings suffered dieback and resprouting.

At the end of 2010, the probability of seedlings growing out of the top of tree shelters was 5.1425 (83.7%) for non-vented tree shelters vs. 2.4234 (70.7%) for vented ones, with an odds ratio of 2.1220 ($p = 0.0416$).

Stem height was significantly influenced by the initial height covariate ($p < 0.0001$); taller seedlings at planting tended to be taller after 2 years. The mulch effect was not significant ($P = 0.2533$) but its two-way interaction with tree shelter type was ($P = 0.0488$). Other significant factors included year ($p < 0.0001$), tree shelter type ($p < 0.0001$) and their interaction ($P = 0.0406$). This interaction resulted from the difference in seedling size between shelter treatments, which increased with time. Total mean height increment over the two growing seasons was 89.7 cm. Most annual growth occurred in the first year with 78.5% (70.4 cm) of growth. Sheltered seedlings were substantially and significantly taller than unsheltered seedlings, for both years of the study. After the first year (2009), seedlings in non-vented and vented tree shelters were significantly taller (94 and 76.5%, respectively) than unsheltered control seedlings (Fig. 4). This difference continued to increase significantly with time, and by 2010, mean stem heights were 99 and 89% greater for seedlings in non-vented and vented tree shelters, respectively, than for unsheltered control seedlings. During this year, there was no significant difference in stem height between sheltered seedlings.

An additional increase in stem height for seedlings inside non-vented and vented tree shelters was recorded with gravel mulch. Lentisk mulch used alone or in combination with Italian stone pine increased stem height only of seedlings in non-vented tree shelters. Italian stone pine mulch resulted in no additional increase in stem height for seedlings in either tree shelter type (Fig. 5A).

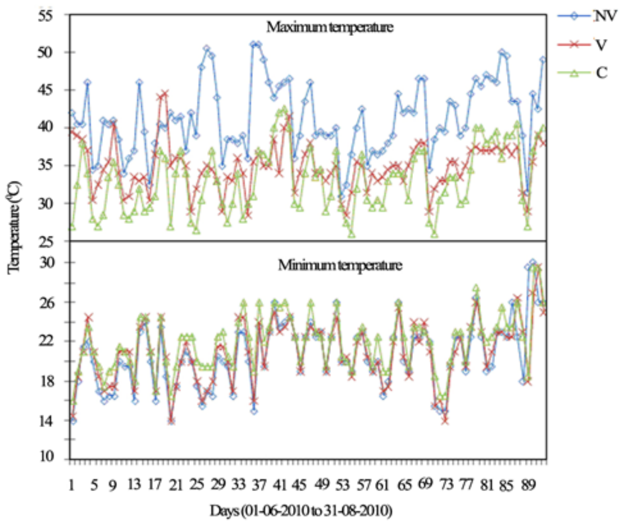

Fig. 3 Maximum (top) and minimum (bottom) daily values of temperature inside (NV, non-vented and V, ventilated) and outside (C, control) the tree shelter, recorded during the summer period from 01-06-2010 to 31-08-2010

Basal diameter

Basal stem diameter at the end of each year varied ranged from 5.6 to 36.1 mm (mean: 14.1 ± 0.29 mm) and from 8 to 58.6 mm (mean: 19.9 ± 0.48 mm), respectively.

Basal stem diameter was significantly influenced by the initial basal diameter ($p < 0.0001$). Other significant factors included year ($p < 0.0001$), tree shelter type ($p < 0.0001$) and their interaction ($p < 0.0001$). Mulch had no effect ($P = 0.0711$), but its interaction with tree shelter type did ($p < 0.0001$). In contrast to stem height, basal stem diameter was significantly greater for unsheltered controls than for sheltered seedlings over the two years of measurement. This difference increased with time. In 2009, basal stem diameters in non-vented (10.9 mm) and vented

(13.5 mm) tree shelters were 38 and 23.2% smaller than in unsheltered control seedlings (17.6 mm), respectively (Fig. 4). By 2010, mean basal diameters for seedlings in non-vented and vented tree shelters were 42 and 30.6% smaller than for unsheltered control seedlings, respectively. During the first and second years, seedlings in non-vented tree shelters exhibited less diameter growth than those in vented tree shelters. Additional increase in basal diameter in vented tree shelters was recorded under the gravel mulch (Fig. 5B). On the contrary, no additional increase in basal diameter was under other mulch treatments for sheltered or unsheltered seedlings.

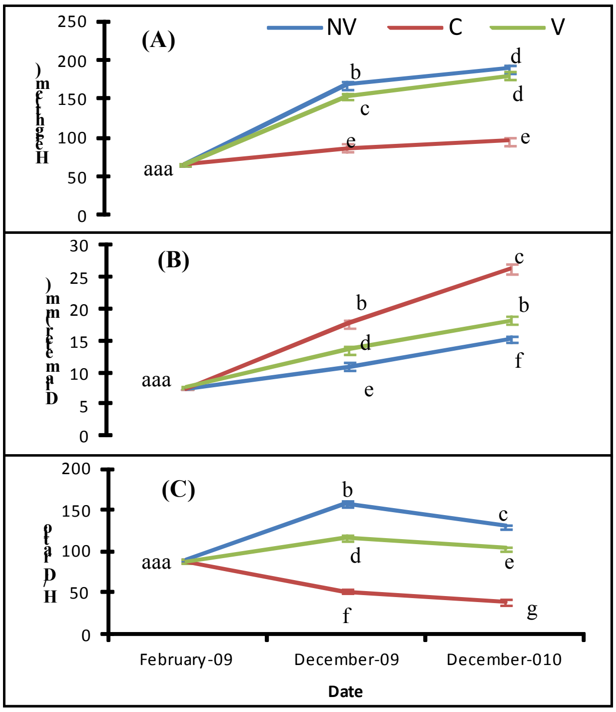

Fig. 4 Height (A), basal diameter (B), and height-to-diameter ratio (*H/D* ratio) (C) according to date of measurement (just after planting: February-2009 and at the end of the 2009 and 2010 growing seasons: December-2009 and December-2010, respectively) and tree shelter type (NV, non-vented; V, ventilated; C, control) and adjusted mean ± S.E. Means marked with different letters within each date were significantly different according to the Tukey–Kramer multiple comparison test, at $P \leq$ 0.05 level.

Height-to-diameter (*H/D*) ratio

The height:diameter ratio was used as an index of structural support provided by the seedlings and was compared to observations of seedling support made at the end of the study. Height:diameter ratios differed significantly by year ($p < 0.0001$). Other significant factors included the initial height:diameter ratio covariate ($p < 0.0001$), tree shelter type ($p < 0.0001$) and its interaction with year ($p = 0.0120$). Compared to 2009, 2010 was characterized by a significant decrease in height-to-diameter ratio, for both seedlings in and out of tree shelters. Over the 2009 and 2010 growing seasons, seedlings in non-vented tree shelters had the greatest height:diameter ratio (taller, thinner seedlings), followed by those in vented tree shelters. The lowest ratio was found in control seedlings (shorter, thicker seedlings) (Fig. 4).

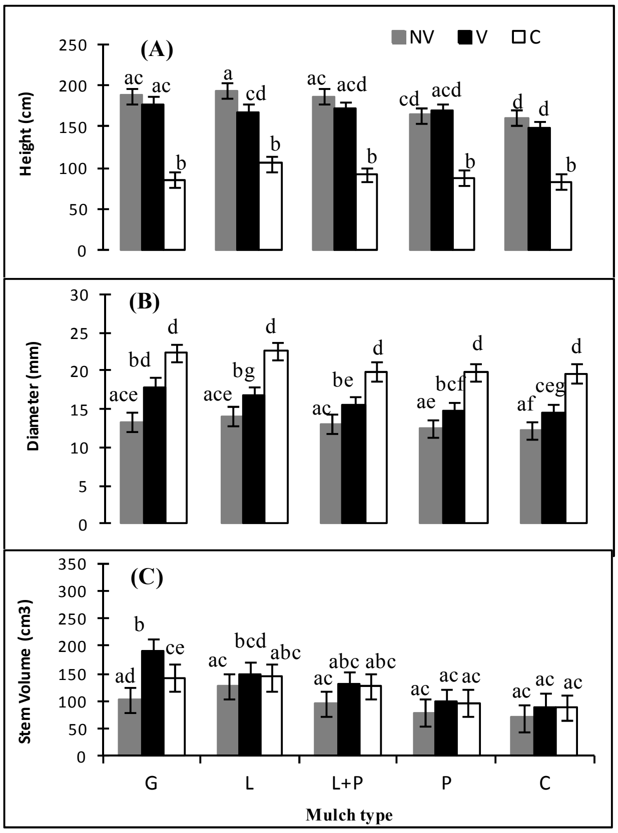

Fig. 5 Height (A), basal diameter (B), and stem volume (C) according to combination of tree shelter type (NV, non-vented; V, ventilated; C, control) and mulch type (G, gravel; L, lentisk; L+P, lentisk + Italian stone pine; P, Italian stone pine; C, control): adjusted mean ± S.E. Means marked with different letters were significantly different according to the Tukey–Kramer multiple comparison test, at $P \leq$ 0.05 level

Stem volume

Stem volume was significantly influenced by year ($p < 0.0001$), tree shelter type ($p < 0.0001$) and their interaction ($p = 0.0288$) in addition to the two-way interaction of tree shelter type and mulch ($p < 0.0001$). Over the 2 years of study, stem volume was higher in unsheltered than in sheltered seedlings. At the end of 2009, the unsheltered control seedlings had the greatest mean stem volume (87.8 cm^3), followed by seedlings in vented tree shelters (85.7 cm^3), but the difference was not significant; seedlings inside non-vented tree shelters had the lowest volume (61.2 cm^3). By the end of 2010, mean stem volume was multiplied by 2.4 for unsheltered seedlings and by 2.1 for sheltered seedlings, with significant differences between all treatments (non-vented and vented tree shelters and control).

None of the organic mulches had an additional effect on stem volume (Figure 5C). However, the gravel mulch produced a 106.4% gain in stem volume, but only for seedlings in vented

tree shelters (92.2 cm^3 for vented tree shelters used alone vs. 190.4 cm^3 when used in combination with gravel mulch).

Polycyclism rate

All seedlings showed rhythmical growth, characterized by a succession of clearly visible flushes. During both years (2009 and 2010), seedlings had one to three flushes. In tree shelters, one flush lasted for 7 weeks: the first 3.5 weeks (average) corresponded to the active growth phase during which a GU was established and the last weeks corresponded to the period of rest. For unsheltered seedlings, one flush lasted for 7.5 weeks, but only the first 2 weeks (average) corresponded to active growth while the last weeks were the rest period. The polycyclism rate was only dependent on year ($p < 0.0001$), tree shelter type ($p = 0.0162$) and year–tree shelter type interaction ($p = 0.0260$). Seedlings were more likely to produce fewer flushes during 2010 than 2009 (odds ratio: 6.0529). Unsheltered seedlings were more likely to produce fewer flushes than sheltered seedlings either during the first year (odds ratio of 2.0302 and 2.7037 for non-vented and control, and for vented and control, respectively) or during the second year (odds ratio of 2.0905 and 2.6002 for non-vented and control, and for vented and control, respectively) (Fig. 6). During both years, seedlings in non-vented and vented tree shelters were more likely to produce about the same number of flushes (odds ratio = 1.3317 and 1.2437, the first and the second year, respectively).

Annual shoot length

Significant factors for annual shoot length were year ($p < 0.0001$), tree shelter type ($p < 0.0001$) and their interaction ($p < 0.0001$), whereas no significant effects of mulch ($p = 0.1403$) and its interaction with tree shelter type ($P = 0.1552$) were found. Overall, annual shoot length increased significantly more in 2009 than in 2010. Moreover, annual shoot length, all polycyclism categories combined, was significantly greater for seedlings in tree shelters than for controls, for both 2009 and 2010 (Fig. 7A). During the first year, annual shoot length was significantly longer for seedlings in non-vented tree shelters, whereas during the second year, seedlings in vented tree shelters caught up and had the longest annual shoots but did not differ significantly from the non-vented tree shelters.

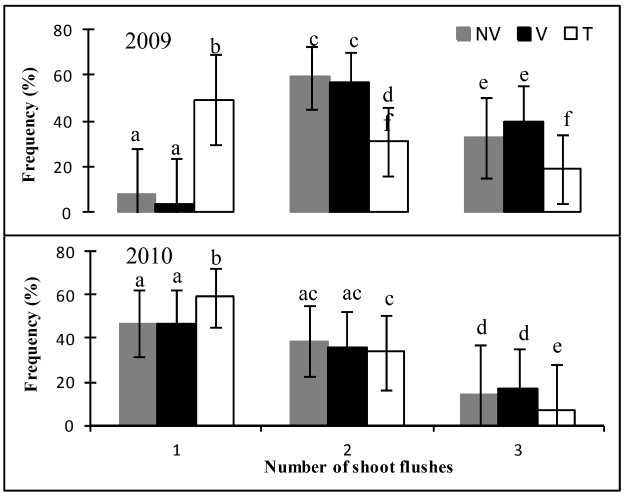

Fig. 6. Frequency (in percent) of the number of flushes (polycyclism rate) established on the main stem according to tree shelter type (NV, non-vented; V, ventilated; C, control), for the years 2009 (top) and 2010 (bottom): percentage ± likelihood confidence limits, at 5% level. Values marked with different letters were significantly different at $p \leq 0.05$ level, according to the χ^2-test.

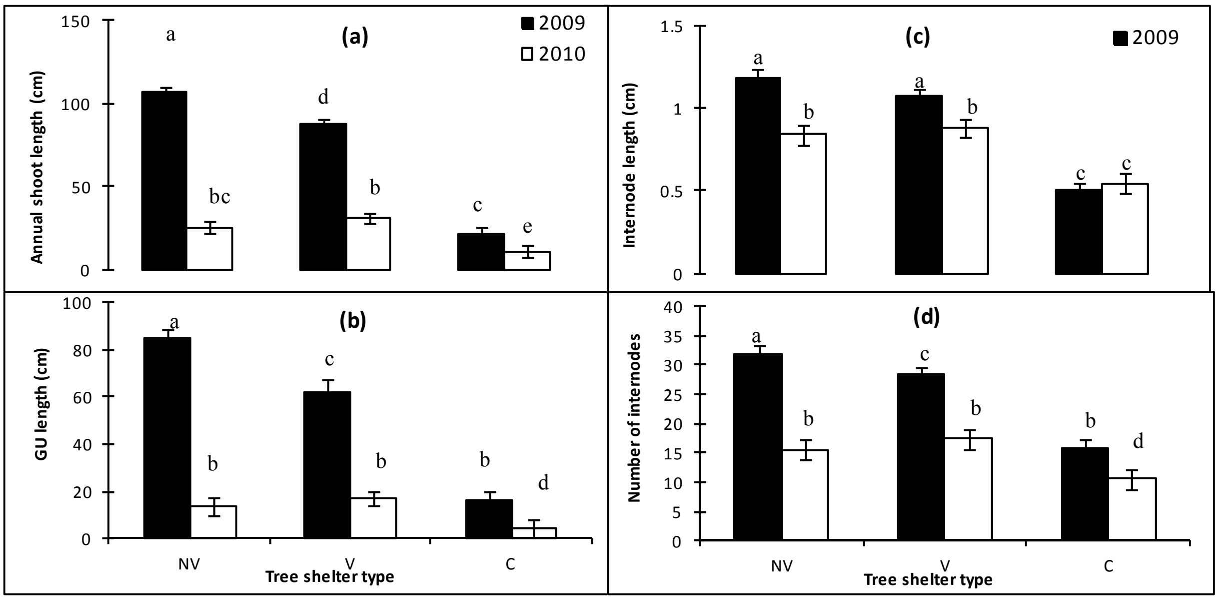

Fig. 7 Annual s(a)ot length (A), GU length (B),(c)ngth (C) and Number (D) of internodes according to tree shelter type (NV, non-vented; V, ventilated; C, control) and growing season (2009 and 2010). adjusted mean ± S.E. Means marked with different letters were significantly different according to the Tukey-Kramer multiple comparison test, at $p \leq 0.05$ level.

GU length

GU length was significantly affected by the negative effect of the number of yearly flushes ($p < 0.0001$) and its two-way interaction with years since planting ($p < 0.0001$); for a given year, the more flushes, the shorter the overall mean GU length. This tendency was less marked in 2010 than in 2009. Moreover, year ($p < 0.0001$), tree shelter type ($p < 0.0001$) and their interaction ($p < 0.0001$) as well as number of flushes ($p < 0.0001$) and its three-way interaction with year and tree shelter type ($p < 0.0001$) had a significant effect on GU length. The latter was not significantly influenced, however, by mulch ($p = 0.4452$) nor by its interaction with tree shelter type ($p = 0.5060$). Overall, GU length was much greater in 2009 than in 2010. Moreover, GU length was significantly longer for seedlings in tree shelters than for controls in 2009 and 2010. Seedlings in non-vented tree shelters exhibited significantly longer GUs than those in vented tree shelters in 2009. During 2010, seedlings in vented tree shelters caught up and exhibited the longest GUs, but not significantly longer than those in non-vented tree shelters (Fig. 7B). When considering the position of the GU on the annual shoot, two different situations were observed: (1) during the first year, GUs of all flushes were significantly longer in tree shelters than in controls, whereas (2) during the second year this was true only for the first flush (Fig. 8).

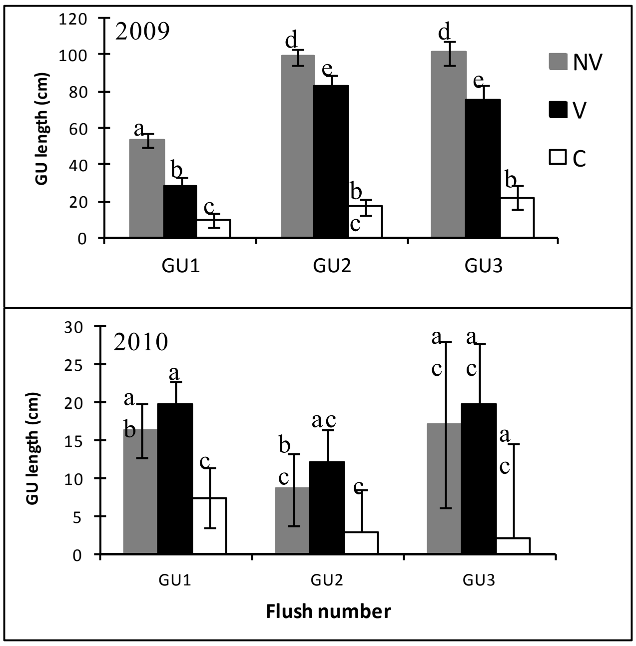

Fig. 8 GUs length according to their number (position) on the annual and tree shelter type (NV, non-vented; V, ventilated; C, control), for the years 2009 (top) and 2010 (bottom): adjusted mean ± S.E. means marked with different letters were significantly different according to the Tukey-Kramer multiple comparison test, at $p \leq 0.05$ level.

Length and number of internodes

Both length and number of internodes per GU were significantly affected only by year ($p < 0.0001$), tree shelter type ($p = 0.0002$ and $p < 0.0001$ for length and number, respectively) and their interaction ($p = 0.0022$ and $p = 0.0004$ for length and number, respectively). For both years, these growth parameters were significantly greater on seedlings in tree shelters (Fig. 7C-D). In 2009, length and number of internodes were both greater on seedlings in non-vented tree shelters than in vented tree shelters. Differences in internode length were not significant. In contrast, in 2010, both length and number of internodes were greater on seedlings in vented than non-vented tree shelters, but the difference was not significant.

Biomass

After two growing seasons, all biomass components were influenced only by tree shelter type. Seedling biomass, expressed as dry weight, showed an inverse trend to that of height, with control seedlings at significantly higher biomass of stem ($p = 0.0017$), branches ($p = 0.0023$), and leaves ($p = 0.0030$) in comparison to sheltered seedlings and consequently a higher total above-ground biomass ($p = 0.0007$)(Table 2). Total below-ground biomass of unsheltered seedlings was also significantly higher than for sheltered seedlings ($p = 0.0005$), both for taproot ($p = 0.0007$) and fine roots ($p = 0.0009$). Root:shoot ratio was also higher for control than for sheltered seedlings but the difference was not significant ($p = 0.5137$). Above- and below-ground biomass were significantly greater in vented tree shelters by 15.8 and 22.1%, respectively, compared with the non-vented shelters.

Number of leaves, leaf area and leaf mass per area (LMA)

After two growing seasons, number of leaves, leaf area and LMA were significantly different only between shelter treatments ($P = 0.0012$, $p < 0.0001$ and $p < 0.0001$, respectively). The mean number of leaves per seedling was 2 times greater for control than for sheltered seedlings. LMA was 22.2 and 23% greater for control seedlings than for those in non-vented and vented tree shelters, respectively. In contrast, mean leaf area was 86% and 100.5% greater for seedlings in non-vented and vented tree shelters than for control seedlings, respectively (Table 2). There were no significant differences between sheltered seedlings for any leaf parameters.

Seedling posture

After two growing seasons, seedling ability to support itself without a stake was only dependent on tree shelter type ($p < 0.0001$). The odds of seedlings with bent posture were 1.2461 (55.4%) in non-vented shelters and 0.6005 (37.5%) in vented shelters, with an odds ratio of 2.0751. Seedling ability to self-support was far greater in vented shelters. No control seedlings showed bent posture. Seedlings with bent posture were taller and had greater basal diameter and height:diameter ratios than erect seedlings (Fig. 9).

Table 2. Dry biomass of stem (SB), branches (BB), leaves (LB), taproot (TR) and fine roots (FR), total above-ground biomass (AGB), total below-ground biomass (BGB), root-to-shoot ratio (RSR), number of leaves (NL), leaf area (LA), and leaf mass per area (LMA) according to tree shelter type (NV, non-vented; V, ventilated; C, control). Mean values ± S.E. Different letters indicate significant differences according to the Tukey-Kramer multiple comparison test, at $P \le 0.05$ level.

	NV	V	C
SB (g)	12.20 ± 2.34 a	17.48 ± 3.09 b	30.80 ± 6.47 c
BB (g)	8.31 ± 1.24 a	9.22 ± 1.27 a	14.70 ± 2.23 b
LB (g)	7.93 ± 1.28 a	6.25 ± 1.06 a	11.51 ± 2.16 b
AGB (g)	28.44 ± 3.23 a	32.95 ± 4.23 b	57.01 ± 7.79 c
TR (g)	11.16 ± 1.04 a	12.51 ± 2.08 a	22.20 ± 3.90 b
FR (g)	2.85 ± 0.28 a	4.60 ± 0.21 b	7.81 ± 1.61 c
BGB (g)	14.01 ± 1.09 a	17.11 ± 2.19 b	30.01 ± 5.33 c
RSR	0.49 ± 0.03 a	0.51 ± 0.05 a	0.52 ± 0.08 a
NL	21.33 ± 32.50 a	13.83 ± 40.83 a	434 ± 40.04 b
LA (cm²)	8.96 ± 0.43 a	9.66 ± 0.43 a	4.82 ± 0.44 b
LM A (gcm^{-2})	0.01068 ± 0.000309 a	0.01061 ± 0.000314 a	0.01305 ± 0.000325 b

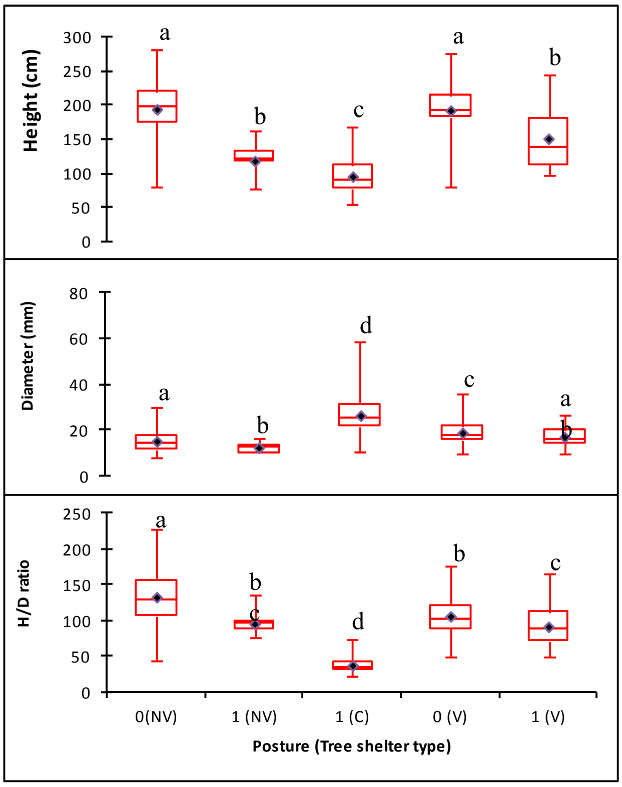

Fig. 9 Box-and-whisker plots for height, basal diameter and height-to-diameter (*H/ D*) ratio according to posture (0, bent; 1, erect) and tree shelter type (NV, non-vented; V, ventilated; C, control) and combination. The box length represents the interquartile range: distance between the 25th and 75th percentiles. The horizontal line and the diamond inside the box indicate the median and the mean, respectively. The vertical lines represent the minimum and maximum values.

Discussion

Tree shelters caused large variations in the growth of *Q. suber* seedlings. After two growing seasons, seedlings initially similar in height and diameter showed substantial and significant differences between treatments. Sheltered seedlings were almost two times taller than controls. Basal stem diameter, however, was smaller for sheltered than unsheltered seedlings. These results confirm previous works that reported tree shelters producing spindly seedlings (Burger et al. 1992; Burger et al. 1996; Jiménez et al. 2005). While growth in height was impressive, sheltered seedlings exhibited reduced stem biomass compared to control seedlings, indicating that the increased height of sheltered seedlings was not the result of an increase in dry matter production. This indicates that smaller stem diameters were not offset by increased height, and confirms the results of Bergez and Dupraz (2000), who suggested that the negative impact of tree shelters on biomass growth was concealed by increased height.

Root biomass was significantly lower in tree shelters. However, there were no significant differences between sheltered and unsheltered seedlings with regard to root:shoot ratio, suggesting that tree shelters did not adversely affect the shoot to root balance. Both diameter and biomass growth were significantly greater in vented as compared to non-vented shelters. This confirms that ventilated tree shelters had some benefit to growth, as reported by Bergez and Dupraz (2000). Seedlings in non-vented tree shelters are subjected to supra-optimal temperatures and varying levels of CO_2 due to poor gas exchange (Bergez and Dupraz 2000), resulting in reduced photosynthesis (Dupraz and Bergez 1999) and carbon gain, leading to altered growth (Hall 2001). Increased air circulation helps to reduce temperature and may increase available CO_2 in vented tree shelters, improving photosynthesis, diameter growth and biomass accretion (Bergez and Dupraz 2000).

The combination of greater height and smaller stem diameters of sheltered seedlings resulted in increased height:diameter ratios and proportions of seedlings unable to support their above-ground mass after shelter removal. Seedlings in vented tree shelters were, however, less likely to exhibit bent posture than were those in non-vented tree shelters: vented shelters enabled more balanced growth either between height and diameter or between shoot and roots. Unsheltered seedlings had the lowest height:diameter ratio and all were self-supporting. These results confirm those of Quilhó et al. (2003) for *Quercus suber*, Jiménez et al. (2005) for *Juniperus thurifera* L., and Burger et al. (1992) and Sharpe et al. (1999) for other species.

Rhythmic growth patterns that characterize cork oak are an endogenous phenomenon (Alatou 1990). Tree shelters merely modulate the endogenous expression of cork oak growth: (1) at the cycle growth level, there were an extension of growth flush and a reduction of the rest period compared with control seedlings, probably due to higher temperatures in tree shelters (Ponder 1994); (2) GUs and annual shoots exhibited significant in-

creases in mean length and length and number of internodes compared with control seedlings, presumably in response to the extended shoot elongation period in tree shelters. These same growth characteristics are also consistent with growth responses that result from shaded conditions (Pemán et al. 2010) and protection from wind stress provided by the shelter (Sharpe et al. 1999). Wind stress on unsheltered plants resulted in reduced shoot and internode length (Neel and Harris 1971). While tree shelters extended the growth flush and contributed to greater development of GUs, a reduction of the rest period gave sheltered seedlings a chance to produce more growth flushes per growing season, resulting, in a marked increase in the polycyclism rate. The greater height of sheltered seedlings in our study resulted from the increase of both the polycyclism rate per growing season and the length of GUs. It should be noted, however, that GU length, in general, tends to be shorter as the polycyclism rate increases. This result confirms the notion derived from previous studies that GUs acquire different properties, such as length, depending on the polycyclism rate (Heuret et al. 2003).

The increase of GU length and polycyclism rate gained from the use of tree shelters enables young trees to grow rapidly in height, which benefits their survival especially in areas where browsing damage is intense (Taylor et al. 2006) or for tree species with relatively slower growth rates.

There was a clear difference in the magnitude of the tree shelter effect on the growth pattern between 2009 and 2010, resulting from variations in annual growth conditions. Precipitation in the 2009 growing season was 953.7 mm as compared with 874.6 mm during 2010. Less rainfall in 2010 might explain the reduction in growth during 2010. Comparison between tree shelters during 2009 showed that GUs of all flushes and consequently, the annual shoot, were significantly shorter in vented than in non-vented tree shelters. By 2010, however, GUs tended to be marginally longer in vented tree shelters and, as a result, the final height was similar for seedlings in both shelter types. During the drier second year, seedlings in vented tree shelters may have been under less stress, perhaps due to a more developed root system, which allowed for increased shoot elongation.

Sheltered seedlings had larger leaves, fewer leaves and reduced specific leaf weight compared to unsheltered seedlings. Famiani et al. (2007) noted the same response in *Olea europaea* grown with tree shelters, and concluded that it was a unique response to low levels of light. Unsheltered seedlings probably had smaller and thicker leaves, while sheltered seedlings had larger and thinner leaves. These morphological adaptations enable shaded seedlings to sustain higher photosynthetic capacity at low light intensities (Hou et al. 2011). However, caution is necessary when shade intolerant species are selected for planting and/or when using relatively dark colored tree shelters where light reductions could reach critical levels for seedling growth (Sharpe et al. 1999; Puértolas et al. 2010).

After two growing seasons, 8.4% of unsheltered seedlings were injured by animal browsing and no longer retained their terminal buds. Sheltered seedlings were not browsed even though >70% had emerged from the top of the tree shelters. This is because sheltered seedlings reached a height where they were less vulnerable to animal browsing. Thus, although planting costs using tree shelters may be relatively high, especially for large reforestation programs, their use may be justified economically and ecologically since they offer protection against animal browsing and promote rapid early height growth, even for a slow-growing species, thus reducing time to establishment (Taylor et al. 2006; Jacobs 2011).

Seedling growth response to mulching could be negative, positive or have no effect, depending on site fertility, the amount of weed competition (Green et al. 2003), mulching methods (Chaar et al. 2008) and the mulch type (Huang et al. 2008). In this study, organic mulches, except Italian stone pine, increased seedling height, while gravel mulch (inorganic mulch) increased diameter and height growth as well as stem volume. It is noteworthy, however, that mulching had no effect whatsoever if seedlings were not protected by tree shelters. This is because the stresses from wind and transpiration, removed by the shelters, are more inhibitive to plant growth than competition for soil resources, which is controlled by mulching (Chaar et al. 2008). Hence, mulch by itself would not be useful in our site conditions. Apparently, wind stress and, to a lesser extent, air circulation through the ventilation holes in vented tree shelters were entirely suppressed inside non-vented tree shelters. As a consequence, additional growth in height was observed with the various mulches. The exception was Italian stone pine, which had no effect on seedling growth. This suggests that alteration of belowground resources related to weed control with this mulch is not likely to have detectable impacts on seedling growth. Seedling growth in height, basal diameter, and stem volume in vented tree shelters was significantly increased only with gravel mulch. This suggests that growing conditions beneath this mulch were improved to a level that enabled seedlings to overcome mechanical stress due to air movement through the ventilation holes. These differences in seedling responses to mulching, under the same site conditions, support the general idea that a beneficial effect of mulching depends on mulch type. Positive effects from the use of gravel mulch have been attributed to increased soil moisture and temperature (Nachtergaele et al. 1998). Conversely, the modest growth observed under organic mulches could be explained by their insulating properties leading to lower soil temperatures (Siipilehto 2001). Benefits linked to organic mulches might be largely associated with their decomposition, which leads to increased nutrient concentrations in soils (Harris 1983). In our study, the mulches were exposed to full sunlight, possibly resulting in lower moisture content throughout the year and lower decomposition rates compared to natural forest litter (Duryea et al. 1999), which might explain the modest gains derived from organic mulches.

Conclusion

Due to their ability to shield seedlings from browsing damage, tree shelters might play a key role in regeneration of cork oak in areas where animal browsing is a concern. While tree shelters might improve regeneration success, their effects on seedling

growth and development might not be entirely beneficial. Although taller seedlings were produced, they had thin stems and smaller root systems that might not support the seedling when the shelter is removed. This problem might be resolved over longer time periods because tree shelters are only removed when the trees outgrow them. Tree shelters are manufactured with a vertical line of laser-generated perforations designed to burst as the tree fills the tube but tree shelter degradation does not always occur even after extended periods (Jacobs 2011). Both non-vented and vented tree shelters were highly effective in (i) protecting young trees against animal browsing and (ii) stimulating growth in height. However, vented shelters provide additional benefits, including improvement of diameter growth and biomass production. Vented tree shelters show promise as an alternative to non-vented tree shelters for producing seedlings with comparable growth height, larger diameter and greater biomass.

Mulching alone (with gravel or organic mulches) was not sufficient to favour seedling growth. Benefits derived from combining the use of tree shelters with organic mulches are not generally encouraging, though an additional increase in height growth in non-vented tree shelters was found under the lentisk mulch when used alone or in combination with Italian stone pine. Good growth results were obtained using vented tree shelters with gravel mulch, resulting in additional increases in height and diameter growth as well as stem volume. With gravel mulch, seedlings in non-vented tree shelters exhibited an additional increase only in height. Thus, a combination of effective tree shelter design and good mulch material is required to obtain improved growth results. Overall, the combination of gravel mulch and vented tree shelters was the best treatment for increasing cork oak height, diameter and volume per tree. Clearly, however, vented tree shelters were the most successful single alternative for the planting of *Q. suber* in this Mediterranean environment. If funding constraints limit managers to applying one ameliorative treatment, vented tree shelter would probably be the best choice.

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